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ORIGINAL PAPER

Biological Synthesis of Gold Nanoparticles by Fungus *Epicoccum nigrum*

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Abstract Gold nanoparticles exhibit unique optical, thermal, chemical and physical properties. The microorganisms have high potential for production of nanoparticles with wide applications. Application of fungi to produce nanoparticles is potentially exciting because of their ability to secrete large amounts of enzymes. In this study, we investigated biosynthesis of gold nanoparticles by the fungus *Epicoccum nigrum* isolated from Andalian gold mine in north-west of Iran. The gold nanoparticles were produced intra and extracellular by reaction of an aqueous solution of chloroauric acid with the biomass of fungus *E. nigrum*. The produced gold nanoparticles were in the size range of 5–50 nm in spherical and rod shapes. This is the first report on the biosynthesis of gold nanoparticles by the fungus *E. nigrum*.

Keywords Nanoparticles · Gold · Biosynthesis · Fungi · *Epicoccum nigrum*

Introduction

Gold nanoparticles have found many potential applications in many fields such as catalysis, sensors, drugs and gene delivery systems [1]. Nanoparticles can be synthesized by physical and chemical methods [2]. The problems of these methods are often experienced with low stability of the nanoparticle preparations, difficult control of the crystal growth and aggregation of the particles [3–5]. One of the most important criteria of nanotechnology is development of clean, nontoxic and

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environmentally acceptable green chemistry procedures [6, 7]. Many biological organisms have known to produce inorganic materials either intra or extracellular [8, 9]. Some well-known examples include formation of iron oxides and various metal nanoparticles using bacteria, fungi or plants [10]. The use of eukaryotic organisms such as fungi in the synthesis of nanoparticles with different chemical composition and size is in progress due to their ability to secrete large amounts of enzymes. The fungi possess some advantages over bacteria. The first is that the most filamentous fungi have a high tolerance towards metals and a high wall-binding capacity, as well as intracellular metal uptake capabilities. In addition, they are easy to culture on a large scale, thus making it possible to easily obtain enough biomass for processing [11]. In an interesting study, Bhambure et al. [11] have reported that the fungus *Aspergillus niger* NCIM616, when placed in a concentrated aqueous solution of $HAuCl_4$, resulted in formation of extracellular gold nanoparticles with the size of 12.79 ± 5.61 nm. In an another recent study, Binupriya et al. [12] demonstrated that the fungus *Rhizopus stolonifer*, when placed in aqueous solution of 1 mM $HAuCl_4$, resulted in the extracellular formation of gold nanoparticles. Here, we report the biosynthesis of gold nanoparticles by a novel metal resistance fungus biomass, *Epicoccum nigrum*, isolated from Andalian gold mine in north-west of Iran. *E. nigrum* is an species of fungus that finds in soil and food. Application of fungi for synthesis of gold nanoparticles has already been reported [11, 12]. However, there is no report on the synthesis of gold nanoparticles using *E. nigrum*. The results showed that this fungus is a good candidate for the simple and clean synthesis of gold nanoparticles.

Experimental

Isolation and Growth of Fungi

Many samples of soil were collected from Andalian gold mine in north-west of Iran in sterilized falcon tubes. Five grams of each sample was diluted with 45 mL of sterile distilled water and placed onto the Sabouraud's dextrose agar (SDA) plates. The plates were then incubated at 27 °C for 5–10 days. All of the isolated fungi were tested for synthesis of gold nanoparticles. Then, the fungus that was found to be able to produce gold nanoparticles was identified based on morphological features, slide culture and growth parameters on malt extract agar or oatmeal agar [13, 14]. The emerged colonies on the petri dishes were purified by single spore isolation. The single spores were maintained on SDA slants at 27 °C. The stock cultures were maintained by subculturing at monthly intervals.

Biosynthesis of Gold Nanoparticles

The isolated fungi were grown aerobically in 250 mL Erlenmeyer flasks containing 100 mL MYPG medium (malt extract 0.3%, yeast extract 0.3%, peptone 0.5% and glucose 1% at pH 5.5–6). The culture was agitated on an orbital shaker (160 rpm) at 27–29 °C for 96 h. The biomass was separated from the culture broth by centrifugation (5,000 rpm) at 10 °C for 20 min. The biomass was washed extensively with distilled water to remove medium components. The fresh and clean biomass (5 g of wet biomass) was suspended in 100 mL of 10^{-3} M aqueous $HAuCl_4$ solution at pH 2.5 in 250 mL Erlenmeyer flasks. The whole mixture was put into a shaker at 27–29 °C for 72 h (160 rpm). The reaction was carried out in the dark.

Characterization of Gold Nanoparticles

The bioreduction of the Au ions in solution was monitored by periodic sampling of aliquots of the aqueous component. The scanning was performed in the range of 400–800 nm by a UV–visible spectrophotometer (Shimadzu, UV Pharma spec 1700 with a resolution 0.72 nm). X-Ray diffraction measurement was carried out using (Philips PW 1800) (XRD). The samples for transmission electron microscopy (Philips model EM 208S) analysis were prepared on carbon-coated copper grids.

Results and Discussion

One out of nine kinds of the isolated fungi was found to be able to synthesize gold nanoparticles. This fungus was identified *E. nigrum*, by morphological features, slide culture and growth parameters on malt extract agar. Colonies of *E. nigrum* are fast growing, suede-like to downy, with a strong yellow to orange-brown diffusible pigmentation. When sporulating numerous black sporodochia (aggregates of conidiophores) are visible. Conidia are formed singly on densely compacted, nonspecialized, determinant, slightly pigmented conidiophores. Conidia are globose pyriform, mostly 15–25 μm diameter with a funnel-shaped base and broad attachment scar, often seceding with a protuberant basal cell; i.e. aleuric or rhexolytic dehiscence of conidia. Conidia become multicellular which divide the conidia into up to 15 cells, darkly pigmented and have verrucose external surface. Pelvinate sporodochia was appearing in 8–10 days, visible as black spots and 100–2,000 μm [15].

By mixing the fungus biomass with the aqueous solution of gold ions, the color of the biomass changed from yellow to purple after 32 h. The color change is a signal for the formation of gold nanoparticles. The reduction of the aqueous chloroaurate ions during exposure to the mycelia was followed by UV–vis spectroscopy [11]. The UV–vis spectra, recorded after completion of the reaction, showed a distinct absorption band centered at 543 nm (Fig. 1) that was very specific for gold nanoparticles.

One of the important ways to characterize nanoparticles is evaluating the XRD spectrum of the sample. The XRD pattern clearly showed that the gold nanoparticles have been formed by the bioreduction of gold ions by the fungus XRD patterns of thin films exposed to $HAuCl_4$, broadening of the intense peaks due to (111), (200), (220) and (311) Bragg reflection at $2\theta = 38.26°$, 44.60°, 64.67° and 77.54°, confirmed the presence of gold nanoparticles (Fig. 2) [9].

The nanostructures synthesized in the fungus cells were further characterized by transmission electron microscopy. The TEM images showed that the gold nanoparticles were formed in spherical and rod shapes both intra and extracellular.

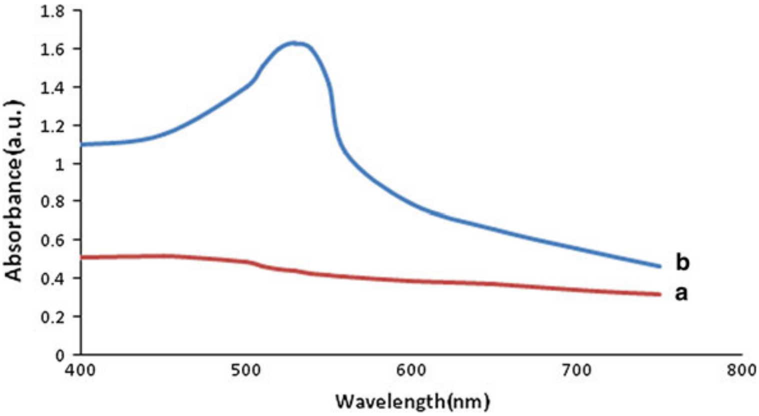

Fig. 1 UV–vis spectra recorded from the fungal cells before (**a**) and after (**b**) exposure to 1 mM aqueous $HAuCl_4$ solution

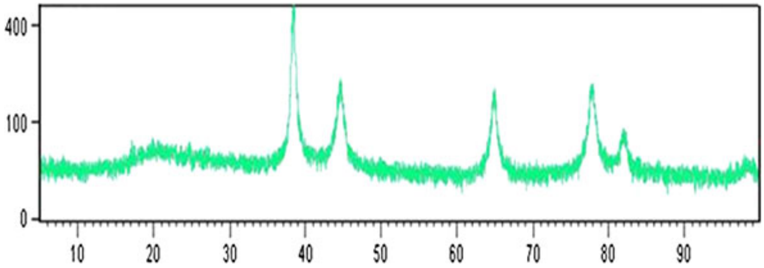

Fig. 2 XRD pattern recorded from an Au nano-*E. nigrum* biofilms

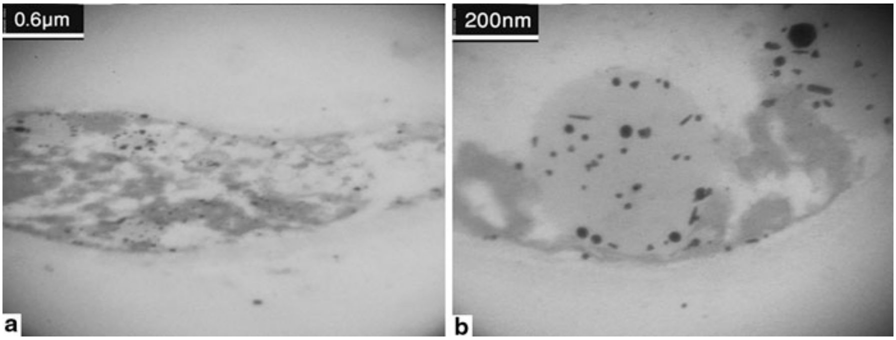

Fig. 3 TEM images at different magnifications of the thin sections of the *E. nigrum* cells after reaction with chloroaurate ions (**a**, **b**). Gold nanoparticles were synthesized both intra and extracellular

The average of nanoparticles size was from 5 to 50 nm. A number of *E. nigrum* cells can be seen in the low magnification of TEM images (Fig. 3). The gold nanoparticles were organized on the cytoplasm and the cell wells.

To our knowledge, this is the first study on the synthesis of gold nanoparticles by *E. nigrum*.

Conclusion

The results showed that the reaction of aqueous chloroaurate ions with the biomass of *E. nigrum* leads to the formation of gold nanoparticles both intra and extracellular.

References

1. S. He, Z. Guo, Z. Yu, and S. Zhang (2007). *Mater. Lett.* **61**, 3984.
2. M. Agnihotri, S. Joshi, K. A. Ravi, S. Zinjarde, and S. Kulkarni (2009). *Mater. Lett.* **63**, 1231.
3. H. Huang and X. Yang (2005). *Colloids Surf. A* **255**, 11.
4. S. Mandal, S. Phadtare, and M. Sastry (2005). *Curr. Appl. Phys.* **5**, 127.
5. D. Mandal, M. E. Bolander, D. G. Mukhopadhyay, and P. Sarkar Mukherjee (2006). *Appl. Microbiol. Biotechnol.* **69**, 485.
6. Z. Ranjbar Navazi, M. Pazouki, and F. Sadat Halek (2010). *Iran. J. Biotechnol.* **8**, 56.
7. I. Maliszewska, L. Aniszkiewicz, and Z. Sadowski (2009). *Acta Phys. Pol. A.* **116**, 163.
8. A. R. Binupriya, M. Sathishkumar, and S. I. Yun (2010). *Colloids Surf. B* **79**, 531.
9. S. S. Shankar, A. Ahmad, R. Pasricha, and M. Sastry (2003). *J. Mater. Chem.* **13**, 1822.
10. P. Mohanpuria, N. K. Rana, and S. K. Yadav (2007). *J. Nanopart. Res.* **10**, 507.
11. R. Bhambure, M. Bule, N. Shaligram, and M. Kamat (2009). *Chem. Eng. Technol.* **32**, 1036.
12. A. R. Binupriya, M. Sathishkumar, and S. I. Yun (2010). *Colloids Surf. B* **79**, 531.
13. R. A. Samson, E. S. Hoekstra, J. C. Frisvad, and O. Filtenborg, *Introduction to Food- and Airborne Fungi*, 6th ed., Chap. 1, p. 118 (Centralbureau voor Schimmelcultures, Delft, 2002).
14. D. H. Larone *Medically Important Fungi—A Guide to Identification*, 3rd ed. (ASM Press, Washington, DC, 1995).
15. D. A. Sutton, A. W. Fothergill, and M. G. Rinaldi *Guide to Clinically Significant Fungi*, 1st ed. (Williams & Wilkins, Baltimore, 1998).